

ASSESSMENT OF GENETIC DIVERSITY IN RICE (*ORYZA SATIVA* L.) GENOTYPES BASED ON INTER SIMPLE SEQUENCE REPEAT (ISSR) GENETIC MARKER

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ABSTRACT

Evaluation of gene conservation strategies, phylogenetic relationship and useful traits in genotype, is essential for the study of genetic variation in the marginal population. In this study, a set of 9 rice varieties were analysed using ISSR markers for genetic diversification. ISSR fingerprinting with 5 anchored primers generated a total 52 scorable loci with an average 10.4 amplicons per primer, out of which 32 were polymorphic (61.53%) and 20 were monomorphic (39.47%). The generated amplicons by each primer varied from 8 to 14 in number. The maximum number of polymorphic bands 10 and 7 were obtained in primers ISSR 12 and ISSR1, respectively. Each primer showed polymorphism ranging from 40 percent to 87.5 percent. The average PIC value ranged between 0.86 (ISSR1) to 0.92 (ISSR12). The genotypes possess 0.57 to 0.96 genetic similarity range. UPGMA based clustering analysis using Jaccard's similarity coefficient grouped these genotypes into two major clusters. Cluster I has the highest number of genotypes (7) and Cluster II (2). This led to a demonstration that for genetic diversity studies in rice, ISSR markers can be used effectively.

KEYWORDS: Genetic Diversity, ISSR, UPGMA, Polymorphism & Similarity

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INTRODUCTION

Rice (*Oryza sativa* L.) is majorly consumed food by more than half of the population around the globe (Nagaraju et al., 2000). It occupies 147 million ha area globally with a production of around 525 million tons. It is majorly cultivated in Asia (Jena and Mackill, 2008) under a wide range of agro-ecological conditions which makes it 'global grain'. In India, about 45% of cereal production is contributed by rice and there are various improved rice varieties. But still, all of these varieties are not effective for specific traits. Hence, still, there is a need for improved varieties in terms of rice productivity and quality, however, broadening genetic base of breeding stocks should be the major aim of the crop improvement program (Vanaja and Babu, 2004). In-plant breeding, the estimation of genetic diversity within the genotypic population is the most crucial step (Rajesh et.al., 2012) which provides the source of genetic improvement.

For the assessment of the genetic variation and relatedness among crop germplasm, numerous techniques are available. DNA based markers are one such technique which is an efficient and reliable tool for analysis and measurement of the genetic diversity in crop germplasm as well as to study phylogenetic relationships and selecting the desired genotype in breeding programs. The molecular markers can distinct the genotypes at the molecular level hence further this species can be ranked as per the number of close relatives and phylogenetic positions they possess, this is an advantageous characteristics of molecular marker over the morphological and biochemical

marker (Rahman et al., 2007). One such molecular marker is ISSR marker, which is based on inter-tandem repeats of short DNA sequences, localized within the microsatellite repeats.

ISSR is great potential markers for analyzing the intra-genomic and inter-genomic diversity by determining the variability between the unique genomic region at several loci simultaneously. These are the random markers hence do not need sequence information, however, it possesses the specificity of sequence-tagged-site markers (Zietkiewicz et al., 1994; Goodwin et al., 1997). The genetic diversity analysis of different rice germplasm makes use of the ISSR markers which are based on AG, GA and (GATA)_n repeats. The ISSR markers are found to be economically reliable and also very informative (Joshi et al., 2000; Davierwala et al., 2000; Sarla et al., 2003, 2005; Reddy et al., 2009).

MATERIALS AND METHODS

Plant Material

Nine genotypes of rice seeds obtained from the Regional Agricultural Research Station, Karjat, Raigad district were used for this study (Table 1). Seedlings of all the rice varieties were grown in the greenhouse. The samples were collected from 15 days old seedlings freshly for the DNA extraction and ISSR fingerprinting.

Table 1: List of the Rice Genotypes used in the Current Study and their Characters

Sl. No.	Genotype	Characters
1	Karjat - 2	Dwarf stature, long slender grain, resistant to blast & neck blast, recommended for shallow and low land areas
2	Karjat- 5	Semi dwarf stature, long bold grain, resistant to neck blast, suitable for midland under rainfed & irrigated conditions
3	Karjat - 7	Dwarf stature, long slender grain, resistant to leaf folder, moderately to brown plant hopper, white brown plant hopper and blast and bacterial leaf blast
4	Ratnagiri-3	Long bold grain, resistant to gall midge, suitable for shallow low land area
5	Ratnagiri-73	Dwarf stature, short and bold grain, moderately resistant to leaf blast, neck blast and bacterial leaf blight
6	Panvel - 1	Semi dwarf stature, short bold grain, resistant to blast & moderately resistant to stem borer, recommended for coastal saline soil of Konkan region, tolerant to salinity condition
7	Panvel -3	Mid tall stature, short bold grain, moderately resistant to blast, stand well in dry spell for more than 17 days, highly salt tolerant suitable for medium to high rainfall area in coastal saline soil condition
8	Palghar -1	Dwarf stature, medium slender grain, moderately resistant to stem borer & blast
9	Palghar- 2	Semi dwarf, short slender grain, moderately resistant to stem borer and blast

Extraction of Genomic DNA (CTAB Method)

DNA extraction was carried out using leaves sample of rice genotypes following the CTAB (Cetyl Tri-methyl Ammonium Bromide) method by Doyle and Doyle (1990) with some modifications. Isolated DNA was quantified using UV visible spectrophotometer (Nanodrop, ND-1000 USA) and visualized on gel documentation unit (Flour Chem. TM Alpha innotech, USA). The stock DNA was highly concentrated so the dilution was made with sterile TE buffer till 50 ng per ml concentration to use as a working solution for PCR analysis.

ISSR Fingerprinting

*(The primers dilution was made in sterilized distilled water with final concentration of 10 pmol/μl).

PCR was performed in 20 μl total reaction mixture with 2μl Taq buffer, 1.6 mm MgCl₂, 200 μM of dNTPs, 0.2 μM primer, 20 ng genomic DNA and 1 unit of Taq polymerase in Corbett Thermocycler. The thermal profile was designed with one cycle of 95°C for 5 min (initial denaturation), 45 cycles of 94°C for 1 min (denaturation), 1 min. at primer specific annealing temperature, 1 min. at 72°C (extension), then 10 min at 72°C final extension. Agarose gel (1.2%) with ethidium bromide was used for the separation of the amplified PCR products by electrophoresis in 1X tris-boric acid-EDTA buffer and were further visualized on a UV transilluminator in Gel Documentation System (Flour Chem. TM Alpha innotech, USA). 1 kb ladder was used for analysing the DNA fragments. The amplifications were performed twice and the results were confirming reproducibility.

Table 2: Details of the ISSR Primers used for the PCR Amplification

Sl. No.	Primer	Nucleotide sequence (5'–3')
1	ISSR 1	(AG) ₈ T
2	ISSR 2	(AG) ₈ C
3	ISSR 12	(AGC) ₅ GA
4	ISSR 17	(AG) ₈ G
5	ISSR 18	(TC) ₈ C

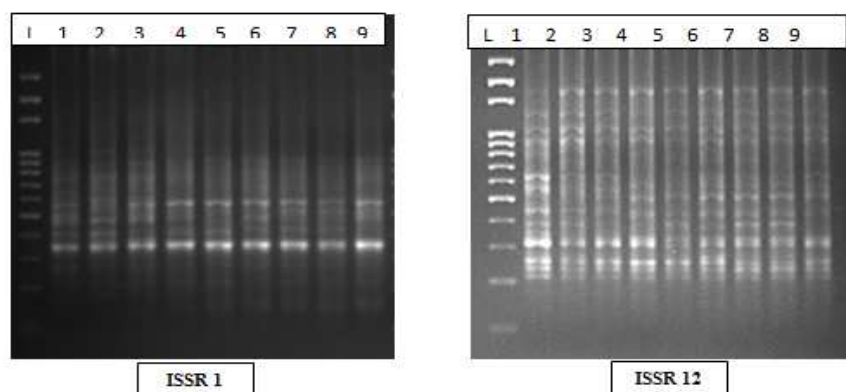


Figure 1: Gel Profile Showing the Amplification of Primer ISSR1 and ISSR 12. The Lane Number corresponds to Rice Genotypes as given in the Table 1, L - 100 bp Generuler.

Table 3: PIC Value, Allele Information and Product Size of ISSR Primer

Sl. No.	ISSR Primer	Total No. of Bands	Polymorphic Bands	Monomorphic Bands	Percentage Polymorphism	PIC Value	Product Size (bp)
1	ISSR 1	8	7	1	87.5	0.8601	300 to 900
2	ISSR 2	11	6	5	54.54	0.8914	180 to 1000
3	ISSR 12	14	10	4	71.42	0.9219	205 to 1750
4	ISSR 17	10	4	6	40	0.8913	175 to 1500
5	ISSR 18	9	5	4	55.55	0.8756	230 to 1150
	Total	52	32	20	61.53		

Data Scoring and Analysis

Amplification profiles of various DNA fragments with different primers were compared for scoring. Depending on the presence or absence of a particular band. DNA fragments were scored manually as (1) or (0) The data analyzed during 1)

Numerical Taxonomy System of Multivariate Statistical Program (NTSYS-pc) software package (Joshi et al., 2000). The dendrogram was constructed using the Unweighted Pair Group Method of Arithmetic Averages (UPGMA).

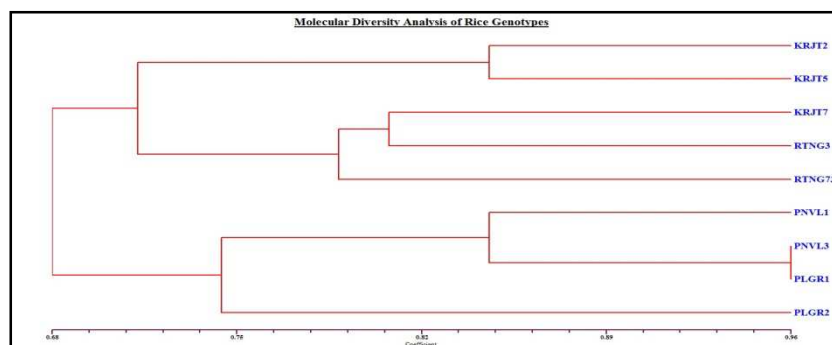


Figure 2: Dendrogram showing Phylogenetic Relationship among Nine Rice Genotype Generated from 5 ISSR Primers.

RESULTS AND DISCUSSIONS

Molecular Analysis using ISSR Marker

The molecular characterization of 9 accessions of rice with 5 ISSR markers which generated 52 Scorable loci with an average 10.4 amplicons per primer, out of which 32 were found to be polymorphic and remaining 20 were monomorphic and shows 61.53 percent polymorphism (Table 3). ISSR primers amplified various DNA fragments and the average range of 4 to 10 polymorphic bands were obtained. The maximum number of polymorphic bands 10 and 7 were obtained in primers ISSR 12 and ISSR1 respectively. The percentage of polymorphism varied with each primer ranging from 40 percent to 87.5 percent (Figure 1). The highest PIC was shown by ISSR 12 (0.92) and the lowest PIC was shown by ISSR 1 (0.86). All five ISSR primers were found to be informative and polymorphic, these primers could be used for diversity analysis which will help to select desirable genotypes in crop improvement programs.

Genetic Similarity among Rice Accession

The binary data derived from amplified bands of ISSR markers were used to obtain a similarity matrix to estimate genetic similarities among nine rice genotypes using Jaccard's similarity indices. The genetic similarity coefficient among various genotypes ranges from 0.57 to 0.96. Wide variation in the similarity coefficient indicated more genetic diversity among the rice accession studied as the genotypes used were from diverse sources. In the study, among the 9 genotypes studied, maximum genetic diversity was observed in pairs of genotypes i.e. Karjat 7 and Palghar1 with a diversity of 42.10 percent. On the contrary, the maximum similarity was observed between Palghar 1 and Panvel 3 i.e. 96.15%. The data of similarity matrices are presented in (Table No. 4).

Table 4: Similarity Index Based on Nei's Genetic Distance, of 9 different Rice Genotypes Based on Variation in ISSR Marker

Genotype	Karjat 2	Karjat 5	Karjat 7	RT 3	RT73	PNVL1	PNVL3	PLGR1	PLGR2
Karjat 2	1.0000								
Karjat 5	0.8461	1.0000							
Karjat 7	0.7115	0.7115	1.0000						
RT3	0.7500	0.6730	0.8076	1.0000					
RT73	0.6923	0.7307	0.7884	0.7884	1.0000				
PLGR2	0.6538	0.6923	0.7500	0.7884	0.6538	0.7307	0.7500	0.7500	1.0000

Cluster Analysis

Genetic diversity analysis was done through the dendrogram obtained by using NTSYS-pc. All the selected ISSR primers were found to be polymorphic in the present study. A dendrogram was constructed to identify the genetic relationship among the 9 diverse genotypes of rice. Based on dendrogram (Figure 4), the genotypes were grouped into two major clusters with 68 percent similarity among them (Cluster-I and Cluster-II). Cluster-I is again divided into two sub-clusters includes Karjat 2, Karjat 5, Karjat 7, Ratnagiri 3 and Ratnagiri 73 with the highest similarity between Karjat 2 and Karjat 5 (84 percent). Subcluster II is also divided into two groups of which the first group includes genotypes Panvel 1, Panvel 3, Palghar 1 and Palghar 2 and among all the rice genotypes the highest similarity is present in Panvel 3 and Palghar 1 (96 percent). However, Palghar 2 was found to be distinct from the rest of the genotypes.

CONCLUSIONS

ISSR primers were found to have a great utility in determining the diversity based on the genotypic constitution of rice, in the fingerprinting of rice genotypes and studying the phylogenetic relationship of these species. The present investigation confirms the earlier studies conducted by Joshi et al., 2000 regarding application of ISSR markers in fingerprinting, analysis of phylogenetic relationship of rice genotypes and on and for distinguishing the traditional and improved rice lines.

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